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(54) Title: APPARATUS FOR OPTICALLY MONITORING CONCENTRATION OF A BIOANALYTE IN BLOOD AND RELATED METHODS

(57) Abstract

Apparatus and an associated method for monitoring concentration of analyte–specific receptors, such as glucose in blood includes a porous hollow sensor body which defines a processing chamber containing a plurality of porous beads, and an analyte, such as glucose–specific receptors bound to helper molecules. A light source causes exciting light to impinge on the sensor body. When glucose is present in a certain concentration within the sensor body chamber, responsive emitted fluorescent light will emerge and may be detected in order to determine the analyte concentration. The analyte–specific receptors may be bound initially to the helper molecules within pores of the beads when the certain level of analyte is not present in the sensor body chamber. The presence of increased levels of analyte in the chamber will cause the analyte–specific receptors to bind thereto and thereby increase the intensity of the emitted fluorescent light thereby providing an indication of the amount of analyte in the blood. When bound to the analyte, the analyte–specific receptors will be positioned outside of the pores in the beads and, therefore, be in more substantial contact with the excitation light. The porous beads are preferably made of opaque material or may be dye–labeled to minimize fluorescent background light levels to minimize detection of the fluorochrome–labeled glucose–specific receptor when the species reside within the porous beads. The invention may be employed in a glucose detector, as well as with other analytes.

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APPARATUS FOR OPTICALLY MONITORING CONCENTRATION OF A BIOANALYTE IN BLOOD AND RELATED METHODS

BACKGROUND OF THE INVENTION

1. Field Of The Invention

The present invention relates to apparatus and an associated method for providing a bioanalyte monitor, such as a glucose monitor, which may be implanted for transdermal use and employs optical means for determining bioanalyte concentration in body tissues. More specifically, it relates to such a system wherein bioanalyte-specific receptors may be bound within the pores of porous beads in the absence of certain levels of bioanalyte in a processing chamber within which they are contained and will emerge from the pores and bind with the bioanalyte when a certain concentration of bioanalyte is received within the chamber.

2. Description Of The Prior Art

Accurate monitoring of bioanalyte levels in patients can be vital to the patient's health. Monitoring of glucose in diabetic patients, for example, is invaluable in order to prevent blindness, kidney diseases, necrosis of nerve tissue, as well as other complications. This will help to improve the quality of life of those people who are affected with diabetes mellitus type I. A highly specific sensor such as a glucose biosensor, if reliable, is widely thought to assume the glucose-sensing function of the beta cells, the natural organ responsible for the glucose level regulation via insulin in healthy humans. Such a sensor, when employed in conjunction with a controllable insulin pump, could function as an artificial pancreas.

On a broader basis, it has become increasingly important in analytical and clinical chemistry to have the capability of remote sensing of chemical and physical parameters. Some methods of performing this type of sensing have been known, such as potentiometry, amperometry, piezoelectric mass determination, conductivity and measurement of reaction enthalpy.

In addition to these methods, optical techniques can be used for remote sensing of analytes and other substances. Optical sensors have certain advantages over electrochemical sensors. For example, optical sensors are immune to electromagnetic interferences. Further, the use of optical fibers can be advantageous when the samples are relatively inaccessible, for instance, in case of in vivo tests. Optical fiber wave

guides allow the transportation of an optical signal over large distances from the sample to an associated meter, for example. Optical fibers can be exposed to varying environments without suffering substantial destruction or deterioration as a result. For a general discussion of sensors and of optical fiber sensors in particular see Wolfbeis, Fibre-optic Sensors in Biomedical Sciences, Pure and Appl. Chemistry, Vol. 59, No. 5 pp. 663-672 (1987). See also U.S. Patents 4,954,318 and 4,999,306.

It has been known to provide optical fiber sensors of various types. The disclosure of U. S. Patent No. 4,334,438, is hereby incorporated herein by reference. This patent relates to a fiber optic sensor having a chamber containing a dialysis membrane which allows selected plasma constituents to pass therethrough and enter the chamber. The chamber contains specific receptor sites in the form of binding agents each of which reversibly binds with one of the plasma constituents. The chamber also contains competing ligands which are dye-labeled. They compete with the plasma constituents for the specific receptor sites on the binding agents. The competing ligands are chosen for their particular optical properties and molecular size so that they do not escape out of the sensor into the bloodstream. The intensity of light emitted from or absorbed by the receptor-site/competing ligand complexes or the free competing ligand alone can be measured by a fluorimeter. This measurement gives a quantitative indication of the concentration of plasma constituents in the blood.

One limitation of the system of U.S. Patent 4,334,438 is that as the fluorescently-labeled compound is bound to the wall, the optical fiber must be inserted exactly straight inside the hollow fiber so that the amount of baseline fluorescence due to the dye-labeled competing ligand bound to the wall is minimized. The glue seams must form a tight seal because with any leak, the chemical constituents of the sensor can escape. The optical fiber within the hollow fiber configuration can also exhibit lack of stability such that any relative movement between the two fibers while in use affects the signal response. In addition, during assembly the proteins which are immobilized are pumped through the fiber under the influence of pressure. This flow method results in variations in the amount of immobilized material along the inside wall, due to variations in the spongy surface causing a variability in the calibration

curves between sensors during manufacture. There remains a need, therefore, for a sensor which overcomes these disadvantages.

Systems such as the one described hereinabove also present another problem. If the device is to be used on a patient, a chronic invasive connection through the skin must be maintained. This can result in a host of problems and annoyances when taking measurements. There remains a need, therefore, for a device that may be used in vivo without the need for chronic connections through the skin.

It has also been known to provide other types of fiber optic sensors. For example, U.S. Patent No. 4,892,383 discloses a fiber optic sensor which includes a modular reservoir cell body and a semi-permeable membrane, however, the sensor requires use of a reagent which precludes reversibility. See also U.S. Patent No. 4,892,640 which discloses a sensor for determining electrolytic concentrations using an ion selective membrane.

United States Patent No. 4,849,172 discloses an optical sensor having a gas permeable silicone matrix that contains a high concentration of an optical indicator consisting essentially of a mixture of derivatives of a polynuclear aromatic compound. U.S. Patent No. 4,857,273 discloses another type of sensor involving enhancement of a light signal response by incorporating a partially reflecting, partially transmitting medium between a coupling structure and an optically dense body.

Optical sensors based on generating a resonance signal in a metallic medium have also been known. See U.S. Patent No. 4,877,747. Other sensors based on detection of refractive index changes in gaseous liquids, solids or porous samples have been known. See U.S. Patent No. 4,815,843 and U.S. Patent No. 4,755,667. Sensors for measuring salt concentrations have also been known. U.S. Patent No. 4,572,106.

United States Patent No. 4,577,106 discloses a remote multi-position information gathering system for obtaining thermometric information from remote locations using fiber optics.

United States Patent No. 4,861,727 discloses a luminescent oxygen sensor using a lanthanide complex. U.S. Patent 4,558,014 discloses assay apparatus employing fluorescence.

Other methods of measuring concentrations of biochemicals in blood include withdrawing blood from the patient for analysis. For example, U.S. Patent No. 3,785,772 discloses a device having a pair of syringes to withdraw blood from a patient, and a dialysis membrane to separate a particular blood constituent from the blood, a reactant which reacts with the chosen blood constituent to form a reactant-blood constituent complex the concentration of which is proportional to the concentration of the blood constituent. This system requires replacement of the reactant after each measurement because the reactant and the blood constituent form an irreversible complex. In addition, the system cannot measure an instantaneous change in the concentration of the blood constituent because of the time taken to remove the blood from the body and obtain a reaction with the reactant.

United States Patent No. 3,638,639 also discloses measurement of blood constituents outside the body. In this system, a catheter is inserted into the blood and lipids are passed through a membrane in the catheter and are dissolved in a solvent which is removed from the body to be analyzed.

United States Patent No. 3,939,350 shows a system for carrying out immunoassays using fluorescence to indicate the presence of a ligand to be detected. An analog liquid is bound to a transparent sheet and contacted with an aqueous assay solution containing the ligand to be detected associated with fluorescent molecules. The ligands become bonded to the sheet and light is passed therethrough to cause fluorescence.

United States Patent Nos. 3,123,866, 3,461,856 and 3,787,119, all disclose means to measure properties of the blood in vivo. These systems comprise optical catheters inserted into the blood for measuring the intensity of light reflected from the blood thereby indicating the blood's oxygen content. None of the aforementioned patents, however, are specifically designed for measuring the

concentration of plasma constituents, such as glucose, in a continuous, reversible manner.

It has also been known to employ oximeters, which are photoelectric photometers, to non-invasively estimate the extent of blood oxygenation. These systems are noninvasive and employ no reagents.

United States Patent 5,143,066 owned by the assignee of the present invention is expressly incorporated by reference herein. It discloses a system for measuring properties of certain substances designated as analytes. A probe housing has an optical fiber associated therewith and has a membrane which is permeable to the analyte being studied. The housing has a reflective surface member disposed between the optical fiber and membrane to define a dark chamber which does not allow light from the optical fiber to enter or exit the chamber. A dye-labeled analog-analyte can pass through the reflective member to permit it to enter an adjacent light chamber where measurements related to the concentration of the analyte may be made. Excitation light from an optical fiber is received within the light chamber. Immobilized receptors are provided within the housing preferably in the dark chamber. The dye-labeled analog-analyte and analyte compete to bind with the immobilized receptors. The dye-containing analog-analyte molecules which do not bind to immobilized receptors pass through the reflective surface member to the light chamber. A light source acting through the optical fiber creates responsive fluorescent light to be emitted by the dye-containing analog-analyte with such responsive light being carried to the detector means. The detector means employ this fluorescent light to determine concentration of the analyte in the sample. In one embodiment, an in vivo sensor which may be placed under the skin is employed. This system, however, employs two chambers alone with fiber optic means and a reflective divider between the two chambers.

U.S. Patent Application Serial Nos. 08/714,830, filed September 17, 1996 and 08/516,257, filed August 17, 1995, which are owned by the assignee of the present invention, discloses apparatus and methods for measuring properties of certain analytes, including glucose. The apparatus includes a sensor capsule having a

processing chamber defined by a wall which has a membrane permeable to the analyte and receptor material disposed within the chamber and capable of chemically interacting with the analyte. In one embodiment, at least a portion of the sensor is translucent. A light source, which may be an optical fiber, causes light to impinge on the translucent portion of the capsule and pass therethrough. Responsive fluorescent light is generated and emitted. Detector means receive and process the light to determine concentration of the analyte. A dye-labeled analog-analyte may be provided within the chamber. The sensor is said to be implantable and can be placed underneath the skin.

Despite these known prior art methods and devices, there remains a need for an improved optical system for providing accurate monitoring of analyte concentration, such as glucose concentration, in a patient's blood. There is also a need for such a system which can function as an optical transdermal system wherein background florescence is reduced so as to facilitate more efficient operation.

SUMMARY OF THE INVENTION

These and other needs have been satisfied by the apparatus and method of the present invention.

The apparatus and method of the present invention preferably provides a porous hollow sensor body which has a processing chamber containing one or a plurality of porous beads that are constructed to block or reduce the emission from a fluorescent dye contained therein and analyte-specific receptors which may be bound to helper molecules located within the porous beads. The porosity of the hollow sensor body is such as to resist passage of the beads, receptors and helper molecules therethrough while permitting passage of the glucose component of the blood therethrough. A light source causes excitation light to impinge on the processing chamber and when no glucose or glucose not reaching a certain level is present emitted responsive fluorescent light will be at a low level. The glucose-specific receptors bound to the helper molecules will be positioned within pores of the beads with the beads preferably being made of opaque material or being dye-labeled so as to minimize florescence background light. When glucose exceeding a certain level is

present in the chamber, the bonds between the glucose-specific receptors and helper molecules will be severed as glucose binds to the glucose-specific receptors and the latter will freely diffuse out of the beads thereby increasing the level of responsive emitted fluorescent light emerging from the sensor body. In a preferred embodiment, the beads will be dye-labeled chemically cross-linked dextran beads and the glucose-specific receptors will be fluorochrome-labeled Concanavalin A and the helper molecules will be dextran.

The porous bead is made of a material that blocks the emission of light from fluorescent dyes when they reside within the bead. When employed as a glucose monitor, glucose-specific receptors are placed within the processing chamber. These fluorescently labelled receptors will be bound within the porous beads in the absence of certain levels of glucose in the processing chamber, and will not respond to excitation when they reside within this region. When a certain concentration of glucose is received within the processing chamber the receptors will emerge from the porous beads and will fluoresce when the processing chamber is exposed to an appropriate excitation light source.

It is an object of the present invention to provide an optical glucose analyte sensor and an associated method wherein background translucent light is minimized so as to enhance the fluorescence response of the sensor in absolute and relative terms to thereby increase accuracy of the analyte concentration determination.

It is a further object of the present invention to provide such a system wherein the system provides a transdermal sensor unit.

It is a further object of the present invention to provide such a sensor which has increased sensitivity and a rapid response time.

It is a further object of the present invention to provide such a system wherein the sensor will be stable over a longer period of time.

It is a further object of the present invention to provide such a system which is reversible responsive to reductions in glucose and reusable thereafter.

It is a further object of the present invention to provide such a system wherein the glucose sensor does not consume glucose.

It is a further object of the present invention to provide a reduction in background fluorescence.

It is yet another object of the present invention to provide a system to facilitate calibration of a glucose sensor.

These and other objects of the invention will be more fully understood on reference to the following description of the invention with reference to the drawings appended hereto.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a partially schematic cross-sectional illustration of a porous hollow sensor for optical glucose monitoring of the present invention.

Figure 2 is a schematic cross-sectional illustration of the beads and associated receptors when the sensor has no glucose present.

Figure 3 is a figure substantially identical to Figure 2 except illustrates the condition of a certain level of glucose being present.

Figure 4 is a plot of absorption and fluorescence versus wavelength for chromophoric components of the particle-based affinity sensor of the present invention.

Figure 5 is a plot of time versus fluorescence with fluorescence given in arbitrary units and the glucose concentration indicated adjacent to the arrows.

Figure 6 is a plot of glucose concentration versus fluorescence response of the sensor.

Figure 7 is a plot of fluorescence response of the sensor versus time in the presence of a predetermined amount of glucose.

Figure 8 is a plot of fluorescence versus time for a sensor of the present invention in the absence of glucose.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "patient" as used herein means members of the animal kingdom including human beings.

Reference herein to a "certain" or "predetermined" amount of glucose being present shall refer to either a specific level desired to be a triggering level in respect of monitoring glucose content or a range of levels employed for such purposes.

Referring to Figure 1, there is shown schematically a preferred embodiment of the present invention wherein a porous hollow sensor body, which may be a hollow body 2 which may be a hollow dialysis fiber, defines a processing chamber 4. The sensor body 2 is, in the form shown, introduced under the skin 6 of a patient, preferably a distance D, which might be on the order of about 0.1 to 5 mm and preferably about 1 to 2 mm. As illustrated schematically herein in Figure 1, the porous hollow sensor body 2 is disposed adjacent to blood vessel 10 and communicates therewith due to the porosity of interstitial tissue. The sensor body 2 contains a large volume of porous beads 14 which occupy about 50 to 80 percent of the volume of chamber 4. Contained within the chamber 4, in a manner to be described herein, in addition to the porous beads 14, are fluorochrome-bioligand glucose-specific receptors 11 which are bound to helper molecules when a certain or predetermined level of glucose 3 is not present in the chamber. The so bound glucose-specific receptors are disposed within pores in the porous beads 14 which are open to the exterior. When the glucose in the chamber 4 exceeds a certain level, the glucose-specific receptors will be displaced from the terminal glucose residues of the beads by the competitive binding of glucose to the binding site of the receptor and the receptors will subsequently emerge from the pores. Binding of the receptor to glucose occurs first and then leakage from the pores takes place. A suitable light source 20, which may be an optical fiber source, causes light beam 22 to impinge upon the porous hollow sensor body 2 which is either translucent or transparent, at least in part, so as to facilitate passage of the excitation light beam 22 through the skin 6 and sensor body 2. This excitation light beam 22 when it impinges upon the glucose-specific receptors bound to glucose will create responsive fluorescent light to be emitted. A photodetector 26, which may be a photomultiplier, receives the emitted responsive fluorescent light beam 28.

It is preferred to have the chamber 4 substantially completely filled with beads so as to minimize gaps of a length significantly larger than the particle diameter which is about 20 to 50 μm.

The photodetector 26 receives the emitted fluorescent light 28 and converts the same into corresponding electrical signals which are delivered to processor 30 which may be in the form of a microprocessor or other computer means. The processor 30 makes a determination of the glucose concentration which may be provided through any output apparatus 32 in hard copy, a visual display, such as a CRT screen, or may be stored on magnetic storage media or in either the processor 30 or output device 32.

In addition sensor body 2 contains another fluorescent dye, 12, which serves to calibrate for intensity of light that actually impinges on the sensor body. The preferred location for the dye is within the sensor chamber 4. Light source 20 provides a light beam 23 that excites the fluorescent dye 12 and causes the emission of light beam 29. Fluorescent dye 12 is chosen so that the color of the light beam is different than light beam 28. The relative intensities of light beam 28 and 29 can be used to compensate for changes in the intensity of light received by chamber 2.

The hollow fiber may have a length of about 0.2 to 1 cm and an external diameter of about 0.2 to 0.3 mm. The ends of the hollow fiber may be sealed with a glue, such as cyanoacrylate, that is activated by water. The geometry of the sensor is not limited to the shape of hollow fibers. Dialysis fibers have shown an excellent history in kidney dialysis, are biocompatible, and have a short diffusion pathway which provides short response time. To improve the fluorescence intensity per unit area, one may align several fibers next to each other horizontally, or use a flat embodiment made up of two semipermeable membrane material (such as regenerated cellulose) layers sandwiching the sensor components in between.

With reference to Figure 2, the porous bead 50 may be of generally spherical configuration and have an external diameter of about 10 to 150 microns and preferably about 70 to 120 microns. The beads 50 have a plurality of pores which are in communication with the exterior surface 52 of the bead 50. When the chamber 4 has either no glucose or a glucose level less than a certain glucose level, the glucose-specific receptors such as 56, 58, 60 and 62, for example, will be bound to the helper molecules and positioned within the pores of the bead 50. As a result, the excitation

light beam 22 which has passed through the patient's skin and the outer wall of the porous hollow sensor body 2 which may be a hollow dialysis fiber, for example, will not create substantial responsive fluorescence.

In a preferred embodiment of the invention, the beads 50 are darkly colored with dyes to establish a broad spectrum light absorption band which includes the excitation and emission wavelengths of the fluorescence label on the glucose-specific receptors. As a result of the condition illustrated by Figure 2, the emission light beam 28 which would normally contain glucose-induced fluorescence be either of small magnitude or non-existent. In lieu of the use of darkly colored dyes on the beads 50, the beads 50 may be made of an opaque material, such as porous carbon, for example.

A suitable bead for use in the present invention is that sold under the trade designation Sephadex G150 which has a high helper molecule density per unit weight of beads. This permits a high number of receptor molecules to be bound to them. Also, the beads can be very efficiently labeled with dyes. This promotes the production of a very high glucose-dependent fluorescence signal. Furthermore, this provides beads which are efficiently labeled with dyes. This provides efficient blockage of light passing into the beads thereby strongly attenuating the background fluorescence of the glucose-specific receptors bonded to the helper molecules and disposed within the pores of the beads. This serves to effect the desired reduction in background fluorescence thereby making the monitor more accurate. Among the suitable dyes which could be used for this purpose are any dyes showing a substantial overlap of their absorption spectra with the excitation and emission spectra of the fluorochrome. For efficient blockage of light when fluorescein and or similar fluorochromes are covalently linked to Con-A the combination of Pararosanilin and Safranin O can be utilized to color the beads. Sephadex beads (a trade designation of Pharmacia, Sweden) are microscopic beads of Dextran, (i.e. the helper molecules) containing terminal glucose residues. The dextran chains are cross-linked to give a three dimensional network.

A suitable glucose-specific receptor is the fluorochrome-labeled "Concanavalin A," designated "Con A," which is bonded to the helper molecule which may be Dextran attached to the beads 50. Con-A is a mannose and glucose-specific protein that is extracted, and affinity-purified from seeds of the plant *Canavalis ensiformis*. It consists of two to four glucose-binding sites. In terms of selecting an appropriate fluorochrome as label for the receptor, fluorescein described herein is only an example. Other fluorochromes, such as Alexa dyes may be beneficial as they have improved photostability and pH-independence. Another aspect that has to be considered is the interference of auto skin fluorescence with the one of the fluorochrome in the wavelength range between 500 and 600 nm. To minimize the contribution of the skin fluorescence to the sensor signal, near-infrared fluorochromes may be preferred. In this wavelength region from 600 to 800 nm skin fluorescence is very low. The dyes attached to the beads to block the fluorescence emission of the receptor should be changed as well, preferably showing a reasonably high absorption coefficient in this aforementioned wavelength region.

Referring to Figure 3, there is shown the bead 50 having a generally spherical outer surface 52. In this figure, the glucose concentration within chamber 4 has reached the desired or certain level and the glucose specific receptors, such as 70, 72, 74, 76, for example, have emerged from the pores of bead 50 and/or bonded to the glucose rather than the dextran helper molecules thereby creating fluorescent emission from the fluorochrome labeled glucose-specific receptors in chamber 4 to product fluorescent emission light beam 80 which is of greater intensity than that shown in Figure 2 and provides an accurate indication of glucose-concentration. It has been found that the fluorescence sensor response time may be on the order of about 300 to 500 seconds.

The relationship between fluorescence as contained in the emission light beam 80 and the glucose concentration is substantially linear from about 0 to 20 mM glucose concentration.

As the skin is a light blocking barrier which will be positioned between the fluorescence detector 26 and the hollow porous fiber body 2, the fluorescence

response of the sensor will be high enough to be measurable and distinguishable from noise. Light may be delivered and withdrawn from the skin by fiberoptic means or by a portable apparatus incorporating a light source, such as a focused laser beam using a lens, and a light modulating and amplifying unit, such as filters and photomultiplier, for example.

Example

In order to verify the effectiveness of the sensors of the present invention and the related method, experiments were performed.

The intensity of the glucose-induced fluorescence signal of a novel particle-based affinity hollow fiber sensor was optimized in order to be able to detect minimal fluorescence changes in a miniature hollow fiber sensor for optical transdermal glucose monitoring. The glucose-sensing bioelements were Concanavalin-A (Con-A) labeled with Alexa 488 (a fluorescein substitute) and dyed Sephadex beads (G150).

The dyes were Safranin O and Pararosanilin which were chemically attached via divinyl sulphone to the beads. This procedure rendered the color of beads red-violet with an absorption peak at 530 nm. The Con A-loaded beads were enclosed into a short segment of a hollow fiber. The beads occupied more than 50 % of the total volume of the hollow fiber lumen. The binding reaction of Alexa 488-labeled Con-A inside the porous beads resulted in a reduction of fluorescence due to the intense light absorption of the dye-labeled beads at the excitation and emission wavelength of 490 and 520 nm respectively. In the presence of glucose, Alexa 488-Con A dissociates from the glucose-residues of the Sephadex beads and diffuses out of the beads into the field of view of the excitation light, leading to large increase in fluorescence (up to 8 times of the background fluorescence). Moreover, the relatively short response time (5-7 min) and the good stability of the sensor over a long period of time (3 month) are excellent, and make the particle-based sensor scheme attractive for in-vivo experiments in rats. The binding reactions used here are shown in equations (1) and (2).

Fluo-Con A+Dextran Matrix$\longleftrightarrow$Fluo-Con A/Dextran Matrix (1)

Fluo-Con A/Dextran Matrix + Glucose ⟷ Fluo-Con A/Glucose + Dextran Matrix (2)

The idea was to hide fluorochrome-labeled Con A inside a light-absorbing matrix from getting excited by the florescence excitation light in the absence of glucose. The matrix is represented by Sephadex beads, which are dyed with chromophores. The absorption spectrum of the chromophore overlaps with the fluorescence excitation and emission spectra of the fluorochrome. In the presence of glucose, fluorochrome labeled Con A is dissociated from the beads and able to diffuse towards the extra-particle space where it can uninhibitedly fluoresce. The performance of the fluorescence-altering binding reaction was tested inside a small segment of a hollow dialysis fiber, the envisaged encasement of the biosensing elements for future in-vivo studies.

The experiments which primarily were targeted at the optimization of signal strength, i.e., generating a maximum fluorescence change in response to physiological concentrations of glucose, and the signal stability over a long period of time under various conditions.

All chemicals were used as received. Divinyl Sulphone (DVS), FITC-labeled Con-A, TRITC-labeled Con-A, FITC-labeled dextran 2000 and Sephadex G 200 (20-50 um) were purchased from Sigma (St. Louis, MO, USA). O Safranin and Pararosanilin were from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). All experiments were done in 12 mM phosphate buffered saline solution (pH 7.1, 0.9 % NaCl, 0.1% NaN_3). Dialysis hollow fibers were from Kunstseidewerk (Pima, Germany). The spectrophotometer (Turner, model 340, Barnstead, Indiana, USA) was used for measuring optical density.

Fluorescence measurements were done with the fluorescence spectrophotometer (LS50B, Perkin Elmer, Beaconsfield, UK). For measuring the fluorescence of Alexa 488-labeled Con-A (particle-based sensor) and fluorescein-labeled dextran (homogeneous sensor), the excitation and emission wavelengths of the spectrophotometer were set at 495 and 520 nm respectively. For the cuvette experiments, the microcell adaptor (Perkin Elmer part No. L225 0139) or the flow-through cell (LC cell accessory, Perkin Elmer part no. L225 0138) were

used. For remote fluorescence measurements, the fiber optic assembly (Perkin Elmer part No. L225 0137) comprising a bundled fiber cable (length 1 m) was utilized.

For the dyeing procedure, the divinyl sulfone method was done according to a method previously reported by *(Porath, et al., (1975) Agar derivatives for chromatography, electrophoresis and gel-bound enzymes. H. Rigid agarose gels cross-linked with divinyl sulphone (DVS), J Chromatography 103, 49-62)*. This method was employed as a chemical linker between the Sephadex matrix and the amine groups of the dyes. Sephadex G150 (250 mg) were pre-swollen in 20 ml distilled water overnight. The beads were washed over a sieve with several volumes of distilled water. The bead suspension (12 ml) was then mixed with 12 ml of a 1 M sodium carbonate buffer solution (Na_2C0_3, pH 11.4) in a beaker. During the entire period of the procedure, the suspension was intensively stirred on a magnetic stirrer. DVS (300 ul) was added to the suspension and allowed to proceed for 1 hour. The beads were washed over a sieve with distilled water to remove non-bound DVS and equilibrated again with sodium carbonate buffer (pH 11.4). Safranin O and Pararosanilin (each 30 mg) were dissolved in DMSO (1 ml). This solution was then added to the bead suspension and allowed to proceed for overnight. Glycine (1 g) was introduced to the mixture to neutralize remaining active DVS groups. After 1 hour the beads were transferred into a 15 ml plastic vial and centrifuged in order to remove non-bound dye molecules. The supernatant was discarded. The beads were re-suspended in DMSO, shaken, and centrifuged again. This procedure was repeated three more times. Distilled water was then used as extraction solvent. When the supernatant became color-free, the violet beads were dissolved in PBS and stored at room temperature.

In fabricating a particle-based hollow fiber sensor, a small volume of bead suspension (100 μl) was mixed with 2 to 3 times the volume of FITC-Con-A solution (see Table 1 for concentrations). After the binding equilibrium was reached, the tube was centrifuged, the supernatant discarded, and 200 μl of PBS was added. Hollow fibers (i.d. 195 μm) were cut to a length of 3-5 cm and glued into the tip of 10-μl pipette tip (Loctite 410, Rocky Hill, CT, USA). This served as a loading

device. The particle suspension was aspirated into a 100-μl pipette tip by means of an adjustable pipette (Oxford, St.Louis, MO, USA) which then was pushed firmly into the opening of the loading device. The tip was pushed firmly into the opening of the tip of the loading device. The thumb knob of the pipette was slowly turned down making the bead suspension flushing slowly into the loading device until the suspension/air meniscus hits the opening of the hollow fiber. The suspension were slightly forced into the hollow fiber by alternately depressing and releasing the thumb knob of the pipette which produced a net motion of the beads moving towards the distal end of the hollow fiber. Sections of the hollow fiber in which the particles were densely packed were cut to a length of 1.5 cm, and rapidly closed with adhesive. The single fiber was introduced into a through flow quartz cuvette of the fluorescence spectrophotometer and its fluorescence response to glucose was measured. A sensor assembly was put together by aligning several single pre-fabricated fibers closely (width and height circa 0.3 cm) along a narrow piece of translucent plastic (width 0.5 cm, length 2 cm), which was then introduced into a polypropylene tubing.

For stability testing purposes, the 10-fiber assembly was introduced into translucent tubing (length 6 cm) which diameter (0.4 cm) was slightly smaller than the width of the assembly plastic base, resulting in a snug fit of the sensor assembly inside the tubing (sensor holder). The sensor holders were stored in PBS buffer under ambient light at room temperature (20-23°C). For the fluorescence measurements, the sensor holder was incorporated into a flow system which was comprised of a waste beaker and pump for pushing glucose solutions through the sensor holder. Right above the translucent sensor holder where the fiber assembly was located, the head of the fiber bundle linked to the fluorescence spectrophotometer was positioned and held with a clamp holder. The optical path length was 2 mm. At this distance, the out-coming excitation light covered the full width of the sensor fibers (core diameter of fiber bundle head 0.4 cm). The whole assembly was covered with black felt to keep stray light out during the fluorescence measurements. The fluorescence change in response to 0 and 20 mM glucose was monitored at various intervals. At the time of measurement, the fluorescence signal in absence of glucose was maximized by slight

changes of the fiber head above the sensor fiber in order to obtain comparative results. The relative fluorescence response, which is expressed as the percentage of the fluorescence change upon 20 mM glucose from the base line fluorescence in the absence of glucose, was normalized as percentage of the initial sensor response at the first day.

Sephadex beads (G150, diameter 20-50 μm) were intensively colored using a combination of two dyes, Safranin O and Pararosaniline. The dyes were chemically attached to the beads via divinyl-sulphone (DVS). The dyed beads showed a broad absorption shoulder at the wavelength of 540 nm (see Fig. 4). (In Figure 4, Curve A is the excitation spectrum of fluorescein, Curve B is the emission spectrum of fluorescein and Curve C is the absorption spectrum of Safranin O and Pararosaniline.) This ensured that the light of excitation and emission of Alexa 488 at 495 nm and 520 nm respectively, were strongly absorbed when Alexa 488-labeled Con-A was bound to the beads. The fluorochrome Alexa 488 has been chosen as a substitute for the more common fluorochrome fluorescein, because it is less prone to photo bleaching and inner-filter effect due to high fluorochrome concentration. The experiments revealed that the color intensity of the beads is a function of the DVS concentration and reaction time. At 1 mg DVS per mg dry weight of beads and after 20 hours of reaction the beads were colored reddish-violet. The binding capacity of the modified beads was 25 mg Con-A per ml wet bed volume twice as high as the original material (11 mg/ml wet bed volume). The increase in binding capacity can be explained by a better accessibility of Con A to glucose residues of the dextran matrix which are usually hidden inside unmodified beads. This result is very favorable for the fluorescence response of the sensor, since it permits to load up the beads with twice as much Alexa 488-Con A as compared to the original material, permitting to measure an even higher fluorescence signal. The decrease in size of those beads had another advantage in terms of filling the hollow fiber and accomplishing a densely packed section of beads inside the hollow fiber. The beads occupied around 70% of the total hollow fiber volume. The efficiency of the dye-labeled beads to quench the fluorescence of bound FITC-labeled Con-A compared to the original, non-labeled

material was measured and calculated to be around 75% at low loading degrees (see Table 1). A typical time-response curve of a 1 week old sensor fiber which was positioned inside the flow-through cuvette is shown in Figure 5. Strong attenuation of the background fluorescence is noted. In the presence of 20 mm glucose, the fluorescence increased up to 4 times. The average time for the fluorescence change in response to glucose was 5 min. In Figure 6 the plot of fluorescence vs. glucose concentration is displayed. The graph is almost linear within the physiological concentration range of glucose (0 to 20 mM). The beads were loaded with different concentrations of Alexa 488-labeled Con-A by exposing them to various feed concentrations to find out the optimal change. It was noticed that the reproducibility of concentration measurements varied depending on the history of the sensor fiber assembly. So when the sensor fiber was stored in PBS overnight, the first glucose measurement yielded a fluorescence change of up to 35% which became smaller with the measurements following immediately after this (10 min) until it leveled off at around 25% of the background fluorescence. The reason for this was that the fluorescence signal no longer reached the original base line.

The change of fluorescence in response to 20 mM glucose was highest at around 0.15 mg/ml FITC-Con-A (see Table 2). The relative exclusion volume of Sephadex G150 beads was estimated to be at circa 40% of the total fiber segment volume which the beads occupied. As a result, the volume accessible to the displaced FITC-Con-A molecules was only around 60% of the total, yielding a molar FITC concentration 1.7 times as high. This translates into the end concentration around 12μM FITC which is based on the molar labeling efficiency of 3.6 FITC per Con-A (Sigma data sheet). This optimal value is in agreement with the optimum fluorescence yield obtained by a fluorescence titration curve of pure FITC-labeled Con-A which showed a saturation at around 11M FITC (data not shown). At a higher lectin concentration (0.35 mg/ml), the relative and absolute change of the fluorescence was slightly decreased due to self-quenching. A molar FITC concentration of 21μM was estimated at this Con-A concentration.

The stability of the fluorescence response upon reaction to glucose was tested over several weeks. Assemblies of 10 hollow fiber segments were prepared which were filled with Sephadex beads and labeled with fluorescein instead of Alexa 488. The fiber assembly was put into a translucent tubing through which alternately phosphate buffered solutions containing 0 or 20 mM glucose were pumped. From the top, the head of a fiber bundle was attached which was linked to the fluorescence spectrophotometer. The particle-based sensor response stayed stable at 100% over a period of 25 days (see Figure 7). Only after that period of time, it dropped down to around 60% where it leveled off until the experiment was terminated (at day 79). By looking at the absolute fluorescence sensor signal in the absence of glucose, a similar picture emerged (see Figure 8). Through the duration of the experiment, the particle-based sensor signal kept fairly stable at around 340 arbitrary units (a.u.). The results indicate a satisfactory operational stability of the particle-based sensor system.

TABLE 1

Efficiency of dye-labeled and non-modified Sephadex beads on quenching the fluorescence emission of bound FITC-Con-A.

Feed concentration of FITC-Con-A (mg/100 μl bead suspension)	Fluorescence (a.u.) Non-modified beads	Dye-modified beads	Quenching efficiency (%)
0.025	28	6	78.6
0.17	231	66	71.4

Sephadex G150 beads were pre-loaded with FITC-labeled Con-A and filled into glass capillaries (W. I mm, length 5 cm). The fluorescence was measured using the head of a fiber bundle linked to the fluorescence spectrophotometer(n=3). The distance from the fiber bundle head to the glass capillary was 3 mm. The background fluorescence which refers to glass capillaries either filled with non-modified or dye-modified beads in the absence of FITC-Con-A, was subtracted from the fluorescence emission data obtained in presence of FITC-Con-A.

TABLE 2

Glucose-induced fluorescence change of the particle-based sensor system as a function of feed concentration of FITC-labeled Con-A. The Con-A loaded beads were encased inside a hollow fiber segment. The ratio of volume of beads to free space was about 3:1. Glucose solutions of 0 mM and 20 mM were alternately pumped through the quartz flow-through cell of the fluorescence spectrophotometer, and the fluorescence response was detected.

Feed conc. of FITC-labeled Con-A (mg/100μl bead suspension)	FITC concentration* (μM/L)	Fluorescence change absolute (a.u.)	Fluorescence change relative (%)
0.025	1	33	17
0.050	2	80	19
0.150	6	120	40
0.350	12	90	37

*based on the labeling efficiency of 3.6 M/L FITC per M/L Con-A (according to data-sheet provided by Sigma)

**refers to the percentage of absolute fluorescence change to baseline fluorescence in absence of glucose 12

It will be appreciated, therefore, that the present invention has provided an improved apparatus and method for optically monitoring the concentration of glucose in blood. It provides a reversible glucose monitor wherein a hollow fiber contains porous beads which receive within its pores glucose-specific receptors bonded to dextran when the glucose in the sensor is at the zero level or below a certain level and the fluorescein-labeled bioligand in the form of the glucose-specific receptors bond to glucose when it is present above a certain level, thereby producing responsive fluorescence to excitation light introduced transdermally and producing fluorescent emission light which can be measured and processed by appropriate sensors.

While for clarity of disclosure reference has been made herein to the preferred use of the present invention in a glucose detector, the invention is not so limited and may be employed to determine concentration in a body fluid, such as blood, other analytes, such as Thyroxine, which is a hormone, Methotrexate, an anticancer agent, Gentamicin, an antibiotic, and Phenytoin, an anticonvulsive, for example, with appropriate helper molecules and receptors as will be known to those skilled in the art.

For simplicity of disclosure, the use of a helper molecule, such as dextran, bonded within the bead pores and a fluorescently-tagged receptor, such as Con-A, bonded thereto for movement in and out of the pore and bonding to glucose. In the alternative, if desired, the receptors, such as Con-A, can be secured within the bond pores and a fluorescently-tagged helper molecule, such as dextran, diffusing in and out of the pores responsive to changes in glucose concentration in the chamber.

Whereas particular embodiments of the invention have been described hereinabove for purposes of illustration, it will be evident to those skilled in the art that numerous variations of the details may be made without departing from the invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. Apparatus for monitoring concentration of a bioanalyte in a body fluid comprising

a porous hollow sensor body defining a processing chamber containing a plurality of porous beads and bioanalyte-specific receptors bound to helper molecules,

said porous hollow sensor body having a porosity which resists passage of said beads, said receptors and said helper molecules therethrough while permitting passage of the bioanalyte component of said blood therethrough,

light source means for illuminating at least a portion of said chamber with excitation light to create responsive emitted fluorescent light therein when bioanalyte concentration exceeds a certain level,

detector means for receiving said light and emitting responsive electrical signals, and

computer means for receiving and processing said electrical signals to effect a determination of glucose concentration.

2. The apparatus of claim 1 including

said bioanalyte being glucose.

3. The apparatus of claim 2 including

said body fluid being blood.

4. The apparatus of claim 3 including

sad porous hollow sensor body being a hollow fiber.

5. The apparatus of claim 3 including

said beads being composed of an opaque material to resist entry of said excitation light into the bead pores.

6. The apparatus of claim 1 including

said glucose-specific receptors being disposed within pores in said beads, and

said pores being opened to the exterior of said beads.

7. The apparatus of claim 3 including

said beads being dye-labeled to resist entry of said excitation light into said pores.

8. The apparatus of claim 6 including

said glucose specific receptors having the property of binding with glucose disposed within said chamber and moving out of said pores when so bonded to increase the amount of said emitted fluorescent light.

9. The apparatus of claim 1 including

at least a portion of said porous hollow sensor body being translucent to facilitate passage of said excitation light and said emission light therethrough.

10. The apparatus of claim 8 including

said beads being chemically cross-linked dextran beads.

11. The apparatus of claim 10 including

said beads having a diameter of about 10 to 150 microns.

12. The apparatus of claim 8 including

said glucose-specific receptors being a fluorescein-labeled bioligand.

13. The apparatus of claim 8 including

said glucose-specific receptors being fluorochrome-linked Concanavalin A.

14. The apparatus of claim 4 including

said beads occupying about 50 to 80 percent of the volume of said chamber.

15. The apparatus of claim 8 including

said porous hollow sensor body being structured to be a transdermal fluorescence sensor for glucose determinations.

16. The apparatus of claim 4 including

said porous hollow sensor body having a length of about 0.2 to 1 cm.

17. The apparatus of claim 12 including

said porous hollow sensor body having an external diameter of about 0.2 to 0.3 mm.

19. A method of monitoring the concentration of a bioanalyte in a body fluid comprising

providing a porous hollow sensor body defining a processing chamber containing a plurality of porous beads and a bioanalyte-specific receptor bound to helper molecules,

providing said porous hollow sensor body with a porosity which resists passage of said beads, said receptors and said helper molecules therethrough while permitting passage of the bioanalyte component of said body fluid therethrough,

impinging excitation light on a translucent portion of said porous hollow sensor body to effect responsive emission fluorescent light when a certain level of glucose is present in said chamber,

detecting said fluorescent light,

converting said light into related electrical signals, and

employing said electrical signals to determine the concentration of bioanalyte in said blood.

20. A method of claim 19 including

said bioanalyte being glucose.

21. A method of claim 20 including

employing blood as said body fluid.

22. A method of claim 21 including

employing as said porous hollow sensor body a hollow fiber.

23. A method of claim 21 including

employing said beads composed of an opaque material to resist entry of said excitation light into the bead pores.

24. The method of claim 20 including

in the absence of a predetermined level of glucose within said chamber maintaining a substantial portion of said glucose-specific receptors within pores in said beads.

25. The method of claim 24 including

resisting entry of said excitation light into said pores by providing dye-labeling on said beads.

26. The method of claim 25 including

said glucose-specific receptors being bound to said glucose within said chamber externally of said beads, and

said glucose-specific receptors bound to said glucose creating emitted fluorescent light responsive to receipt of said excitation light.

27. The method of claim 26 including

said beads being chemically cross-linked dextran beads.

28. The method of claim 19 including

employing said porous hollow sensor body as a transdermal glucose sensor by inserting said body under the skin of the patient.

29. The method of claim 28 including

positioning said porous hollow sensor body about 0 to 5 mm under the surface of the patient's skin where the emitting light will be introduced.

30. The method of claim 27 including

employing as said beads, beads having a diameter of about 10 to 150 microns.

31. The method of claim 27 including

employing as said glucose-specific receptors a fluorescein-labeled bioligand.

32. The method of claim 31 including

employing as said glucose-specific receptor fluorochrome-labeled bioligand Concanavalin A.

33. The method of claim 24 including

introducing said beads into said chamber in such a manner that said beads occupy about 50 to 80 percent of the volume of said chamber.

34. The method of claim 27 including

employing said porous hollow sensor body having a length of about 0.2 to 1 cm.

35. The method of claim 34 including

employing said porous hollow sensor body having an external diameter of about 0.2 to 0.3 mm.

36. The method of claim 20 including

employing as said helper molecules, molecules which are fluorescently-tagged.

37. The method of claim 36 including

said helper molecules being bound to said glucose within said chamber and moving out of said pores when so bonded.

38. The method of claim 37 including

immobilizing said glucose-specific receptors within said bead pores.

39. The method of claim 31 including

immobilizing said helper molecules within said bead pores.

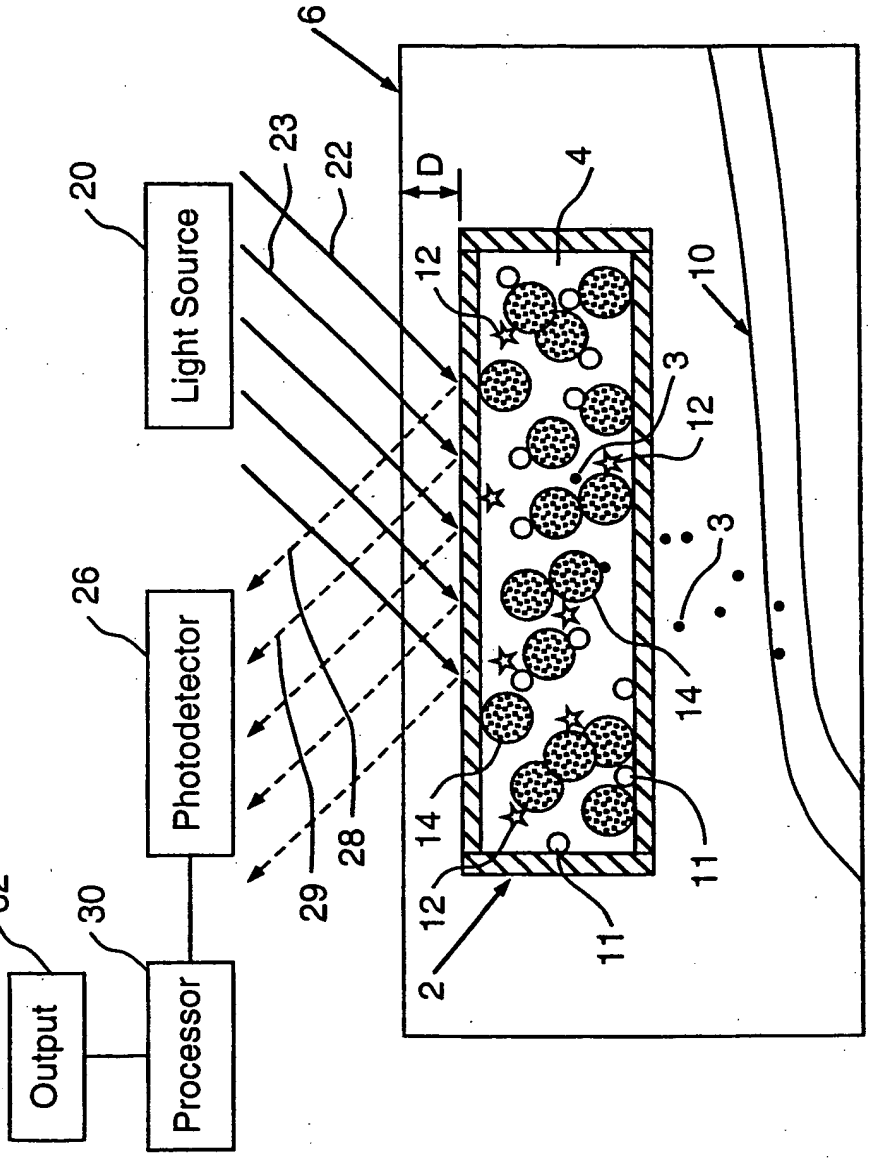
Output
Processor
Photodetector
Light Source
32
30
26
20
6
23
22
D
4
12
10
3
12
3
14
14
12
2
11
11
29
28

FIG. 1

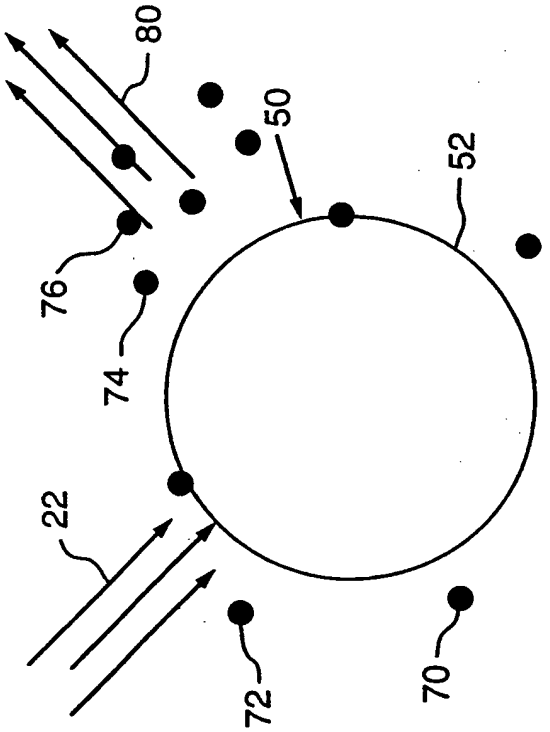

FIG. 3

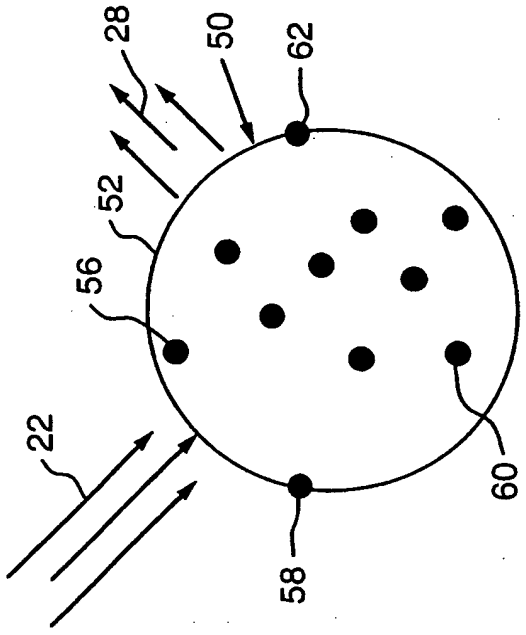

FIG. 2

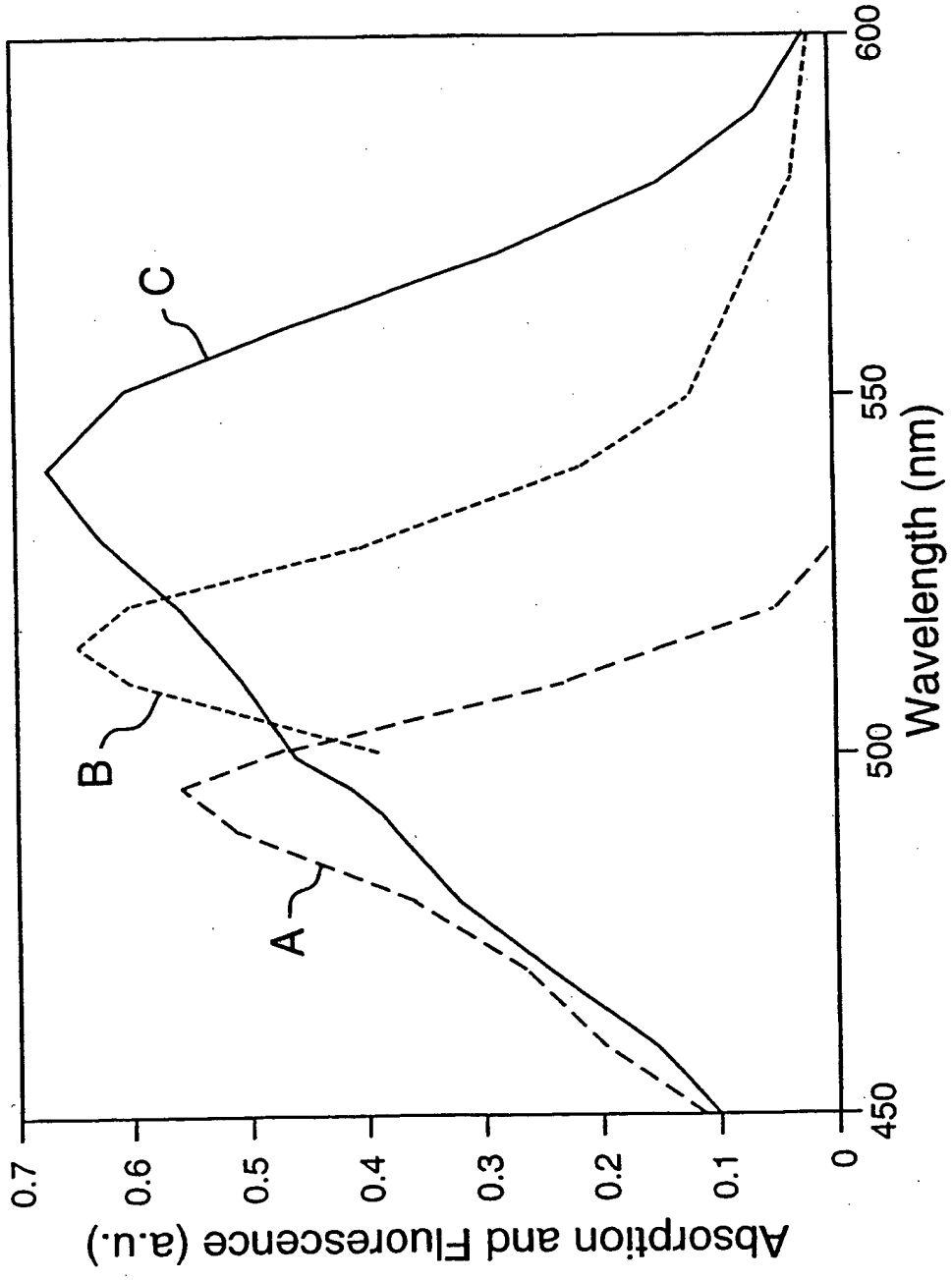
Absorption and Fluorescence (a.u.)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
450
500
550
600
Wavelength (nm)
A
B
C

FIG. 4

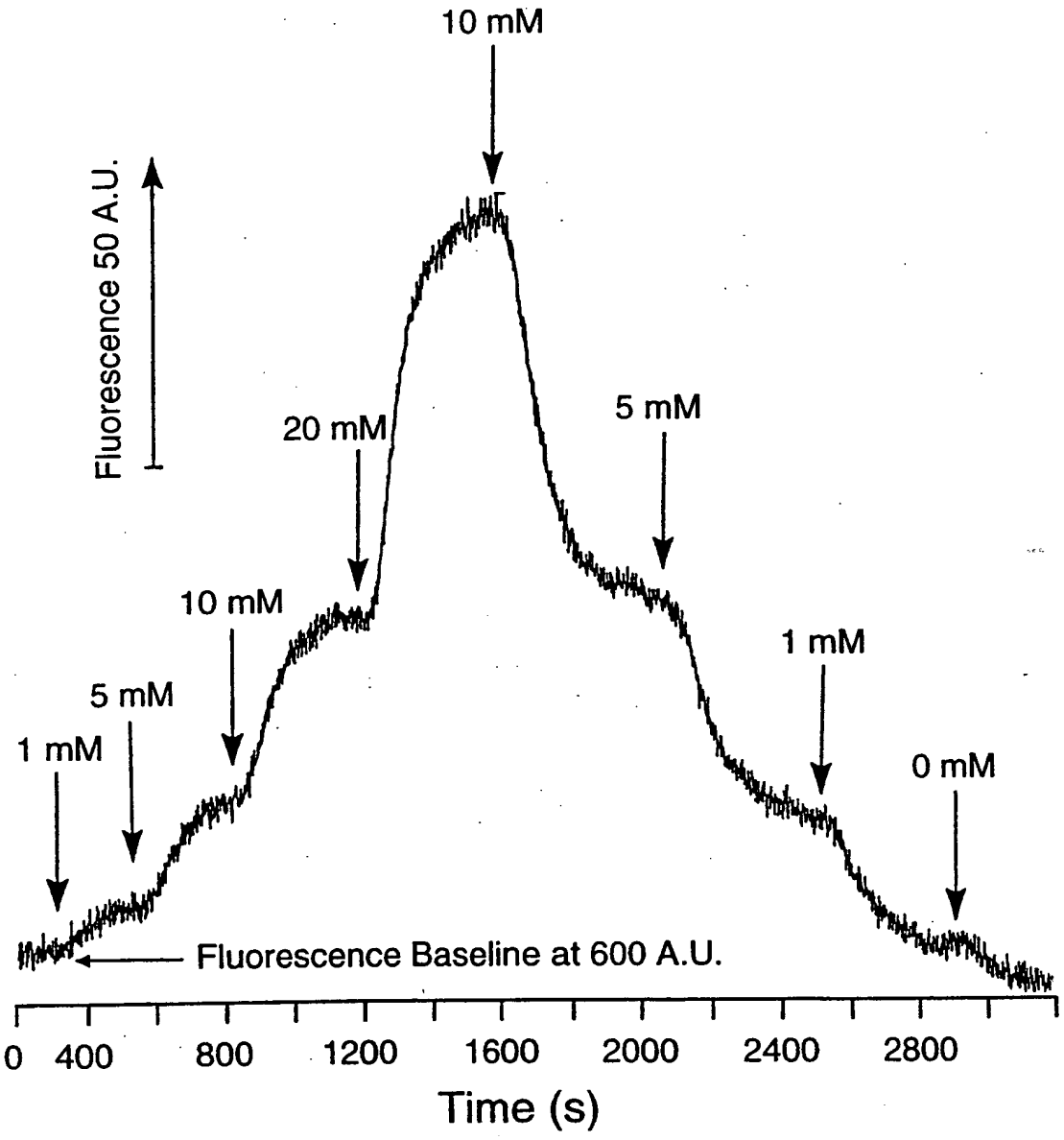
10 mM
Fluorescence 50 A.U.
20 mM
5 mM
10 mM
1 mM
5 mM
1 mM
0 mM
Fluorescence Baseline at 600 A.U.
0
400
800
1200
1600
2000
2400
2800
Time (s)

FIG. 5

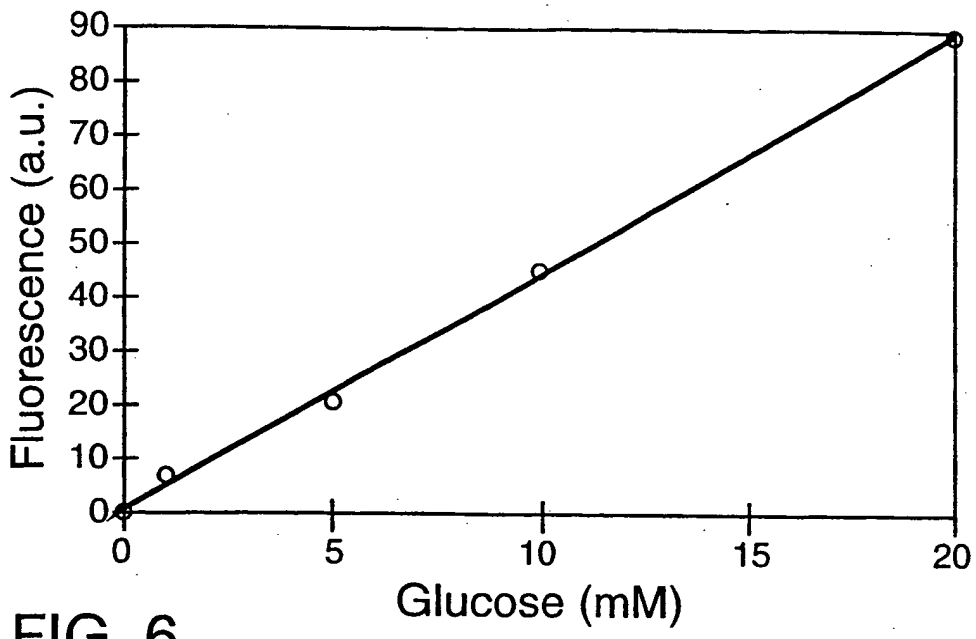
Fluorescence (a.u.)
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
Glucose (mM)

FIG. 6

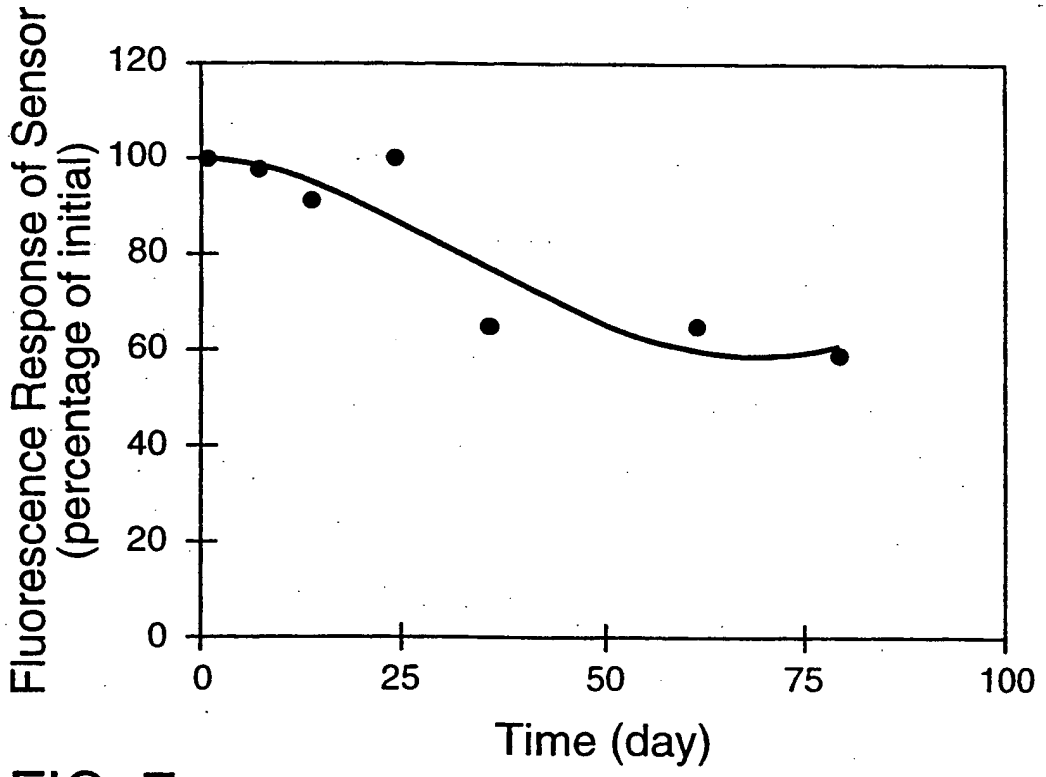
Fluorescence Response of Sensor
(percentage of initial)
120
100
80
60
40
20
0
0
25
50
75
100
Time (day)

FIG. 7

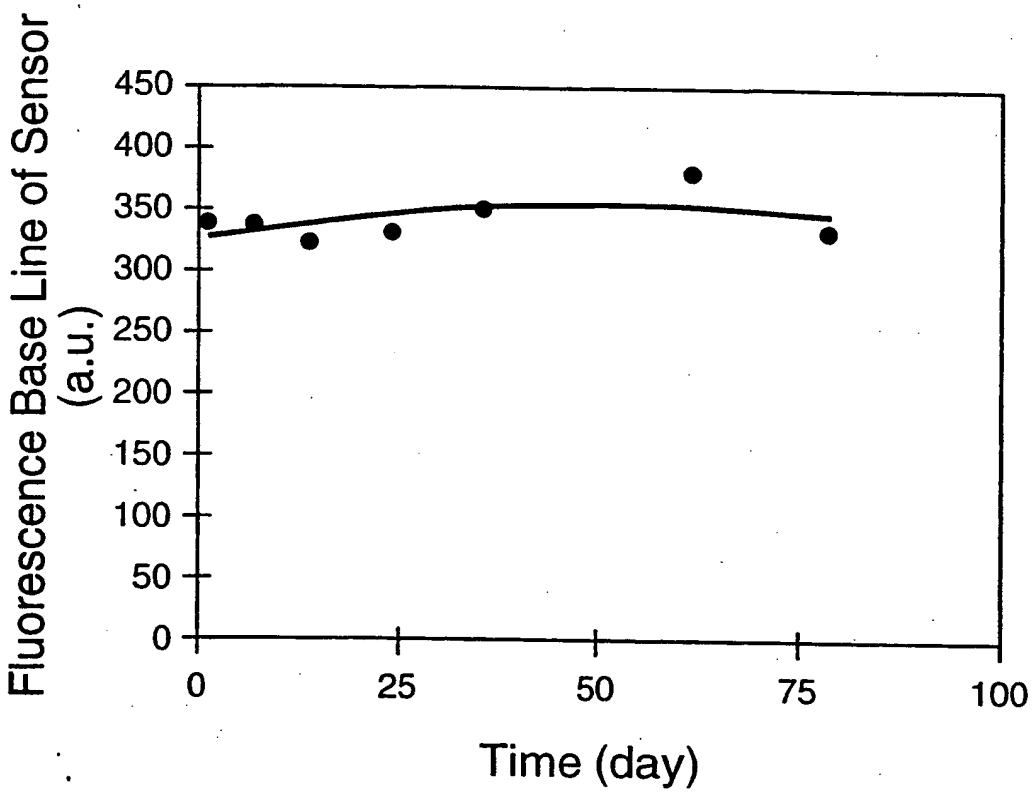
Fluorescence Base Line of Sensor (a.u.)
450
400
350
300
250
200
150
100
50
0
0
25
50
75
100
Time (day)

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/11268

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 51/00; C12Q 1/00, 1/54; G01N 21/64, 21/27

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 378/ 44; 422/ 82.06, 82.07, 82.08, 82.09; 424,1.21, 1.29; 435/4, 14; 530/396; 600/348, 360; 607/2;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, EAST, WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3,492,396 A (DALTON et al) 27 January 1970, see whole document.	1-5, 7,9, 11, 14,16-17,19-23, 30, 34-37
Y	US 4,344,438 A (SCHULTZ) 17 August 1982, see whole document.	6, 8, 10, 12-14, 24-29, 31-33, 38-39
A	US 4,954,318 A (YAFUSO et al.) 04 September 1990, see whole document.	1-39
Y	US 5,143,066 A (KOMIVES et al) 01 September 1992, see whole document.	1-5, 7, 9, 11, 14, 16-17, 19-23, 30, 34-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 JULY 2000	Date of mailing of the international search report 02 AUG 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JANA HINES Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/11268

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

378/ 44; 422/ 82.06, 82.07, 82.08, 82.09; 424,1.21, 1.29; 435/4, 14; 530/396; 600/348, 360; 607/2;